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The universality of bioenergetic disease and amelioration with redox therapy

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Abstract

Overt mitochondrial diseases associated with mitochondrial DNA mutations are characterized by a decline in mitochondrial respiratory function. Similarly, a progressive decline in mitochondrial respiratory function associated with mitochondrial DNA mutations is clearly evidenced in aged human subjects. This communication is concerned with the development of a rat model for the study of bioenergy decline associated with the ageing process and overt mitochondrial diseases. The model involves the treatment of young rats with AZT to induce skeletal and cardiac myopathies. It has shown that there is a decline in soleus muscle function in vivo and that this decline is mirrored in the capacity of heart sub-mitochondrial particles to maintain bioenergy function. Coenzyme Q_{10} and several analogs were administered with AZT as potential therapeutics for the re-energization of affected tissues. Coenzyme Q_{10} and especially decyl Q were found to be therapeutically beneficial by both in vivo improvement in soleus muscle function and in vitro cardiac mitochondrial membrane potential capacity. Sub-mitochondrial particles were also prepared from heart mitochondria of young and aged rats. The particles prepared from the aged rats were found to have a decreased ability to maintain membrane potential as compared to those derived from the young rats.

Keywords: Mitochondrial DNA; Mutation; Bioenergy; Coenzyme Q (ubiquinone); AZT

1. Introduction

The occurrence of overt mitochondrial diseases (myopathies) associated with mitochondrial DNA (mtDNA) mutation was first recognized by the laboratory of Morgan-Hughes in 1988 [1]. Over the next few years (1988–1991), there was a rapid identification, albeit of apparently rare occurrence, of overt mitochondrial diseases associated with mtDNA mutation and mitochondrial bioenergy deficiency. They acquired exotic diagnostic labels, including Leber's hereditary optic neuropathy (LHON), chronic progressive external ophthalmoplegia (CPEO), Kearns Sayre syndrome (KSS), myoclonus epilepsy with ragged red fibers (MERRF) and mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS). These hitherto uncommon diseases have now come into the mainstream of medicine with increasing numbers running into thousands being identified yearly since their features have become better recognized.

A striking feature of the pathological mtDNA mutations is what might be considered their classical nature; the range and types of mutations associated with these human diseases have all been described several decades earlier as mutations occurring in yeast mtDNA [2]. These mutations range from large mtDNA deletions, characteristic of the yeast petite mutants, now recognized as commonly associated with the KSS and CPEO syndromes. Mutational changes in the mtDNA encoding proteins of the oxidative phosphorylation complexes, denoted *mit*$^-$ mutations, have been described by many laboratories working on yeast mitochondria, and these types of changes are now described as associated with human syndromes such as LHON. Mutations in the tRNAs, so called *syn*$^-$, are characteristic of MERRF and MELAS. From these overall considerations in 1989 Linnane and associates formulated the hypothesis that these types of mtDNA mutations will occur randomly and accumulate with age, leading to a progressive decline in the bioenergy capacity of tissues/organs [3]. This bioenergy decline was suggested to contribute significantly to the etiology and pathology of a range of age-associated degenerative diseases and to the general frailty of very advanced age. The degenerative

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diseases would now be envisaged to include heart and vascular conditions, various encephalomyopathies, Parkinson's disease, some types of adult onset diabetes mellitus and others.

Linnane and associates in 1989 also included [3] the concept that tissue bioenergy mosaics will develop with age due to the random mutation and turn-over of mtDNA in post-mitotic tissues (muscle, brain, others) and also the segregation of the mitochondrial genome in mitotic tissues. The concept of tissue bioenergy mosaic entails the representation in tissues of a mosaic of cells containing mitochondria with different bioenergy capacity. In such a mosaic there will be a range of bioenergy capacity amongst cells in which some cells will have a high bioenergy capacity through to cells which will be severely energy deficient. These considerations and our subsequent data have lead to the concept of the Universality of the Bioenergetic Disease [4].

Linnane and associates [3] also suggested that the oxidative phosphorylation system of tissues could be partially re-energized by means of redox therapy, whereby specific redox substances, such as coenzyme Q_{10} (Q) and others, could ameliorate the consequences of the age-associated decline in electron transport function which is responsible for the decline in bioenergy capacity. At this time, the tissue energy mosaic has been confirmed by several laboratories using enzyme histochemical techniques [5–7], although these studies do not specifically demonstrate that mtDNA mutations contribute to the bioenergy mosaic; putatively, the involvement of nuclear DNA mutations could produce the same effect. However, recent work of our laboratory with in situ PCR techniques clearly demonstrates that the tissue bioenergy mosaic is strongly correlated with the tissue distribution of mtDNA mutations (Baumer, A. and Linnane, A.W., in preparation). In this paper we shall focus on recent developments of redox therapy with Q analogs, at the levels of intact laboratory animals, skeletal muscle performance and bioenergetic properties of isolated mitochondria.

2. Results and discussion

We have experimentally approached the problem of mitochondrial bioenergy decline and re-energization both at the physiological and molecular levels. AZT (zidovudine), the main drug of choice in the treatment of AIDS, is well known to induce in humans and rats severe skeletal and cardiac myopathies [8,9]. AZT inhibits mitochondrial DNA polymerase and consequently, prolonged treatment of patients or animals with AZT induces profound changes in structure and function of mitochondria which are reminiscent of the pathological changes observed in a number of mitochondrial diseases and ageing. We have used AZT treatment of rats administered by intraperitoneal injection for the induction of myopathies as a model for the study of

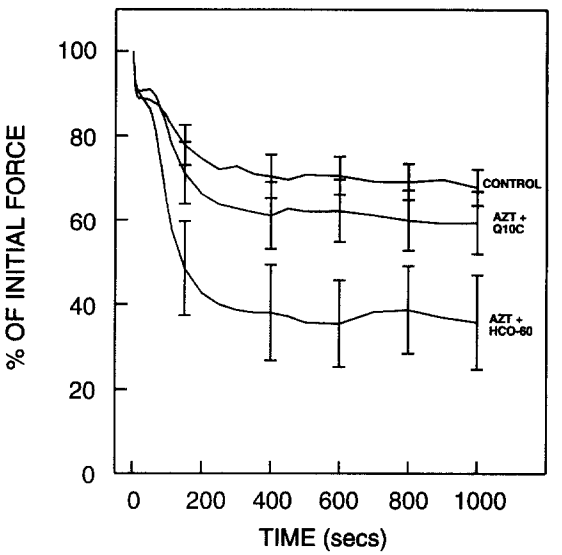

Fig. 1. In vivo mean fatigue profiles of rat soleus muscle. Three groups of 6 month old female rats (8–9 animals each) were treated daily with i.p. injections for 85–90 days as follows: (i) control group, injected with the surfactant HCO-60 (0.2 ml of 2% aqueous solution); (ii) a group injected with AZT (10 mg/kg body weight/day) dissolved in 0.9% NaCl and simultaneously injected with HCO-60; (iii) a further group injected with the same concentration of AZT as in (ii) as well as Q_{10C} (decyl Q) (see text) dissolved in 0.2 ml of 2% HCO-60. The mechanical force of the stimulated soleus muscle in vivo was recorded for a period of 1000 s as described (see text and [10]); the bars indicate the data averaged among all the animals of the particular group.

the progressive age-associated bioenergy decline of tissues and the subsequent re-energization of the tissues with Q analogs, both at the whole animal and the isolated mitochondria levels. We also briefly describe the decline in bioenergy function of heart mitochondria isolated from aged rats as compared to young rats.

Soleus muscle, a major muscle of the hindlimb, was chosen for the in vivo studies because it contains 85–90% type I fibers and 10–15% type IIA fibers. Type I fibers are slow-twitch with a high mitochondrial density and are normally highly resistant to fatigue; type IIA fibers are fast-twitch with a relatively high mitochondrial density and are also fatigue resistant. The performance of the soleus muscle was investigated in vivo in the anaesthetized rats (urethane); the muscle was surgically exposed and electrically stimulated via its nerve with the muscle blood supply maintained intact. Recording conditions were isometric with the muscle set at optimum length and distally attached to a strain gauge [10]. The fatigue profile of the muscle was determined by stimulation at 40 Hz for 330 ms every second (i.e. duty cycle one third of a second stimulated, two thirds rest) for periods up to 8000 s. The contractile responses and the muscle electromyographic (EMG) signal were recorded on tape and records captured on line with a computer based logging system.

Experiments with the soleus muscle of young adult rats (Fig. 1) have shown that the muscle can maintain its force at approx. 70% of the initial force for over 8000 s (the data

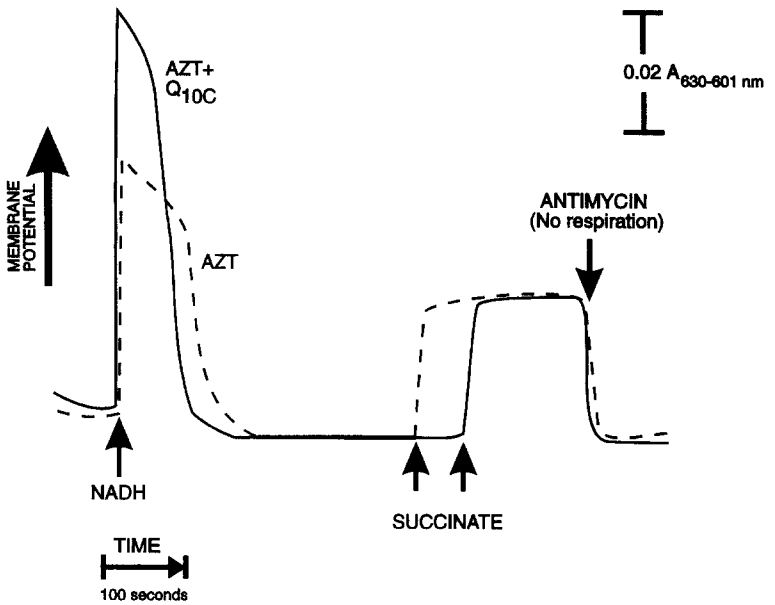

Fig. 2. Membrane potential of rat heart ETP_H isolated from AZT-treated animals. Sub-mitochondrial particles ETP_H (electron transfer phosphorylating) were prepared from the frozen hearts of rats from the same groups used in the experiments of Fig. 1. Each ETP_H preparation was derived from approximately four pooled rat hearts. Membrane potential was monitored at 630–601 nm with 3 μM oxonol VI [12] in an Aminco-Chance dual wavelength spectrophotometer at 25° C with 0.17 mg/ml of particles, as described [13]. The dashed trace was obtained with particles prepared from animals treated with AZT, whereas the solid trace from animals treated with both AZT and Q_{10C} as in Fig. 1. The concentration of NADH and succinate were 0.1 and 20 mM, respectively. Antimycin (1 μM) was added to collapse the membrane potential completely. The times of addition of these inhibitors are indicated by vertical arrows.

shown is for 1000 s of stimulation). With such a stimulus regime, the EMG signal did not alter significantly after the first 150–200 s indicating that this initial reduction in muscle force was not attributable to failure of action potential propagation in either the muscle or the nerve or to neuromuscular failure. Young rats (2 to 3 months old) were subjected to AZT treatment administered by intraperitoneal injection at the rate of 10 mg/kg body wt. per day for up to 90 days, a dose comparable to that therapeutically used in human patients. Treatment with AZT clearly decreases the steady state level of soleus force performance (Fig. 1). The level of soleus muscle force that is

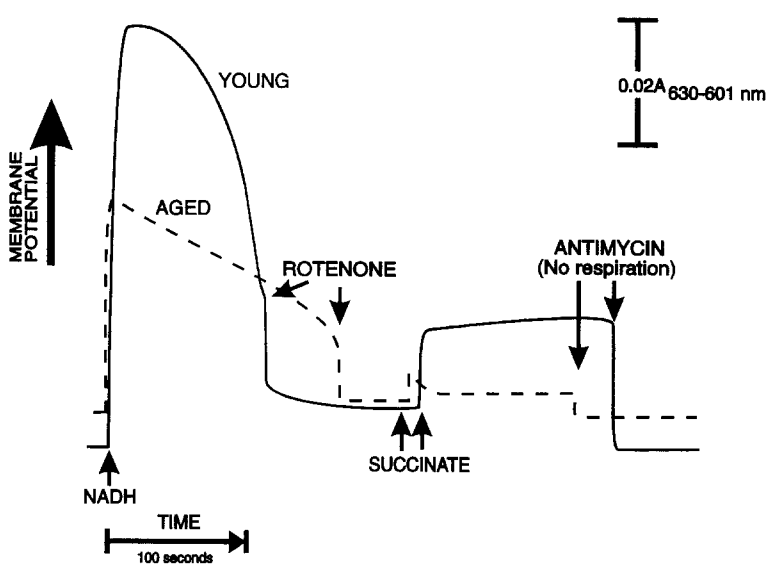

Fig. 3. Membrane potential of mitochondrial ETP_H prepared from the hearts of young and aged rats. Each preparation was derived from four pooled rat hearts from animals of similar ages: young rats (5–6 months old), aged rats (34–36 months old). Conditions were identical to those in Fig. 2. The concentration of particles was adjusted to give the same rate of succinate: Q reductase activity (complex II) for each pooled preparation. Rotenone was added at a final concentration of 1 μM to completely block the membrane potential generated by NADH. Note the time scale of the traces is shorter than that in Fig. 2 with all other indications as in Fig. 2.

maintained after the (150–200 s) initial decrease is about 40% of the initial value. Several groups of rats in addition to receiving AZT were simultaneously injected with either coenzyme Q_{10} or its analog propenyl-Q (2,3-dimethoxy-5-methyl-6-propenyl benzoquinone, Q_{3C}) at a rate of 2 mg/kg body wt. per day, or decyl-Q (2,3-dimethoxy-5-methyl-6-decyl benzoquinone, Q_{10C}) (0.75 mg/kg per day). As shown in Fig. 1, muscle performance was essentially normal with those rats receiving AZT plus Q_{10C}. Although not shown in the figure, coenzyme Q_{10} was not as effective as Q_{10C} in restoring muscle performance, while Q_{3C} had no significant effect on improving the muscle functional decline as induced by AZT treatment. This is the first documented evidence that redox therapy with Q analogs can successfully ameliorate the energetic performance of whole tissue in vivo after mitochondrial damage induced by AZT.

The observed decline in soleus muscle function induced by AZT treatment and the restoration of the muscle performance by simultaneous Q_{10C} treatment can be directly correlated with an improvement in mitochondrial bioenergy function. Previous studies have shown that AZT preferentially accumulates in skeletal and cardiac muscle of rats after prolonged treatment and that the induced morphological changes in the mitochondria of both tissues is the same [9]. Heart mitochondria are more stable and robust than skeletal muscle mitochondria, therefore we have chosen to perform the enzymological studies in heart mitochondria isolated from the frozen heart tissue of the same rats utilized in the muscle physiology studies.

ETP_H (electron transfer phosphorylating sub-mitochondrial particles) were prepared from heart mitochondria [11] isolated from AZT-treated rats and AZT plus Q_{10C} treated rats. The membrane potential generated by NADH oxidation in the two preparations was measured with the optical probe oxonol VI [12] as recently described [13]. The particles from the AZT plus Q_{10C} treated rats clearly have a higher membrane potential than those rats treated with AZT alone (Fig. 2). The membrane potential signal from the AZT plus Q_{10C} treated rats was similar to that measured in particles from untreated rats (data not shown). There was no observed difference between the two groups of rats in the membrane potential as generated by succinate oxidation (Fig. 2) indicating that complex I is the major site of the bioenergy damage induced by AZT treatment. Parallel studies (again data not shown) demonstrate that the electron transport activity of complex I is the most affected of the respiratory complexes by AZT treatment and can be restored with Q_{10C} administration. These in vitro data mirror those found in the in vivo soleus muscle function.

Fig. 3 compares the membrane potential of heart ETP_H prepared from young (5–6 months) and aged (34–36 months) rats. The membrane potential generated by NADH and succinate is dramatically reduced in the particles prepared from aged rats as compared to those prepared from young rats. In the experiment shown in Fig. 3 the concentration of the particles prepared from young and aged rats were adjusted to have the same rate of complex II activity. Consequently, the data shown in Fig. 3 serve to emphasize the marked differences in the mitochondrial membrane potential generation of young and aged rats. The results obtained with aged rats are in notable contrast to those obtained in young rats treated with AZT (cf. Figs. 2 and 3); the AZT effects are restricted to complex I whereas in the aged rats complexes III and IV are also involved in the bioenergy decline of the mitochondria.

Acknowledgements

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